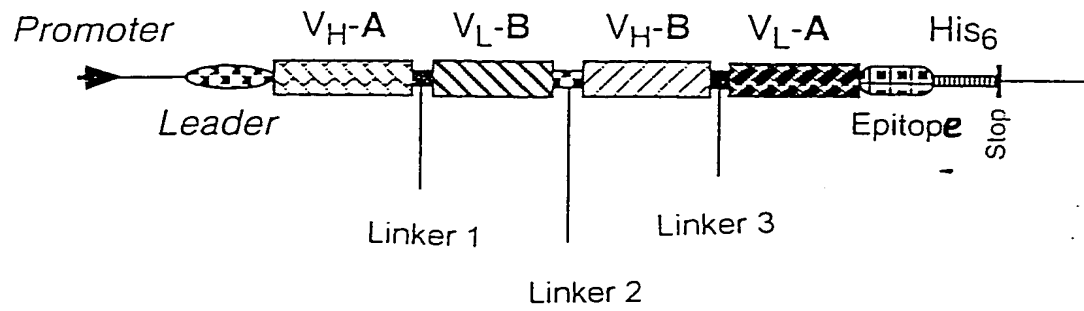
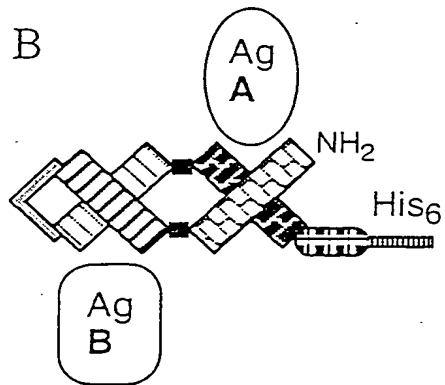


A



B



C

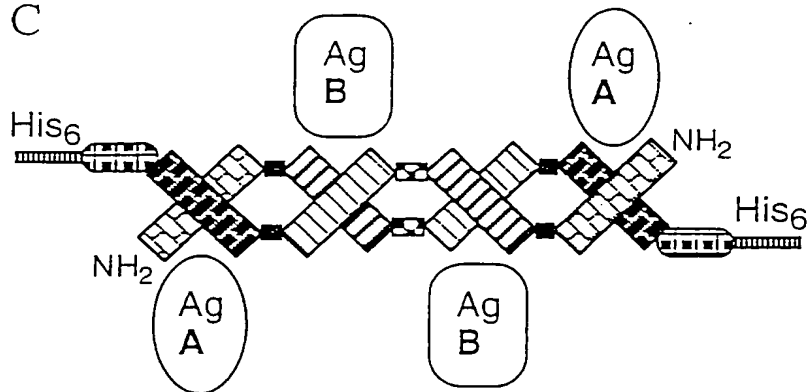
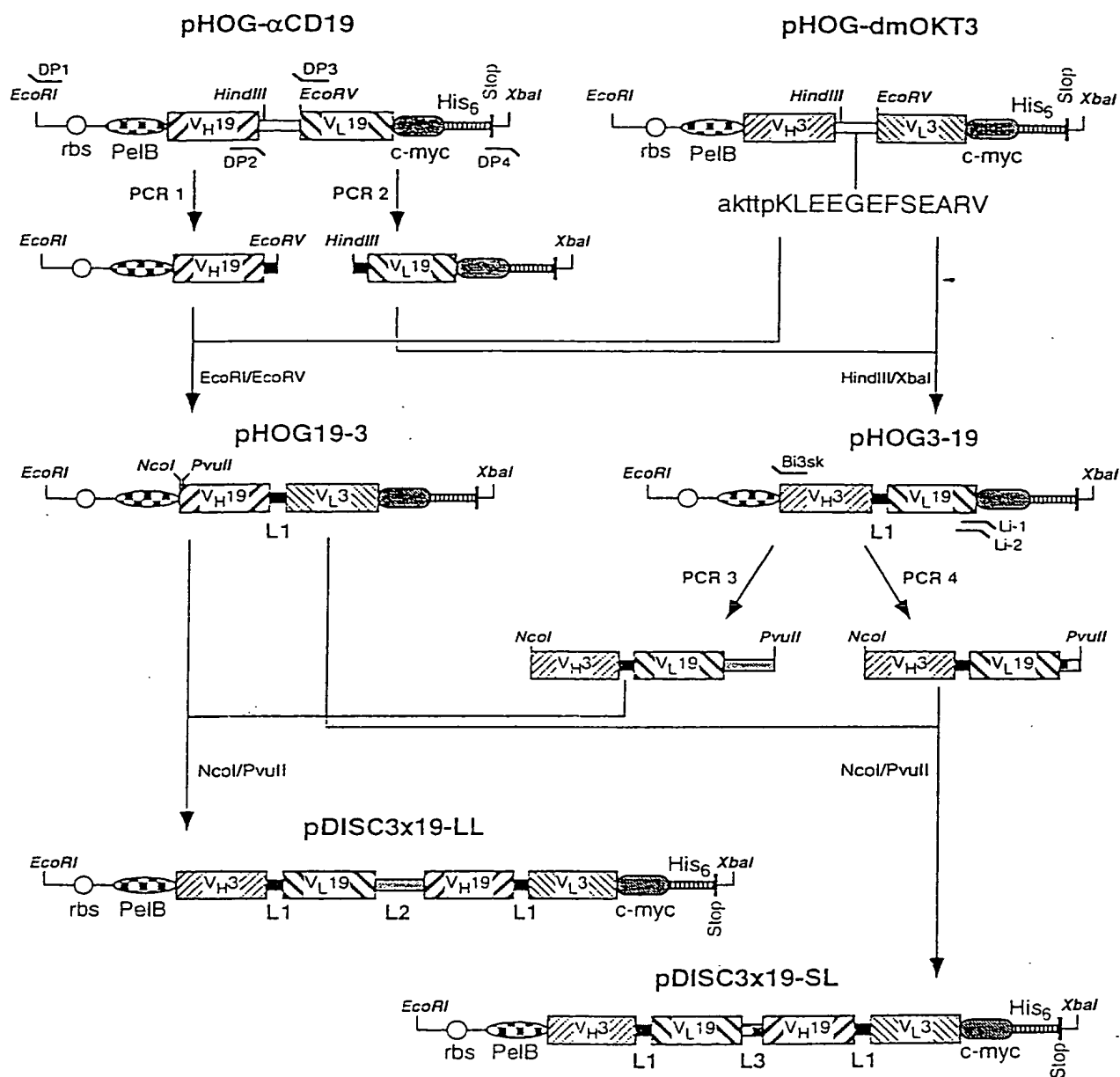


FIGURE 1



Linkers: L1 = GG
 L2 = (G₄S)₄
 L3 = GGPGS

FIGURE 2

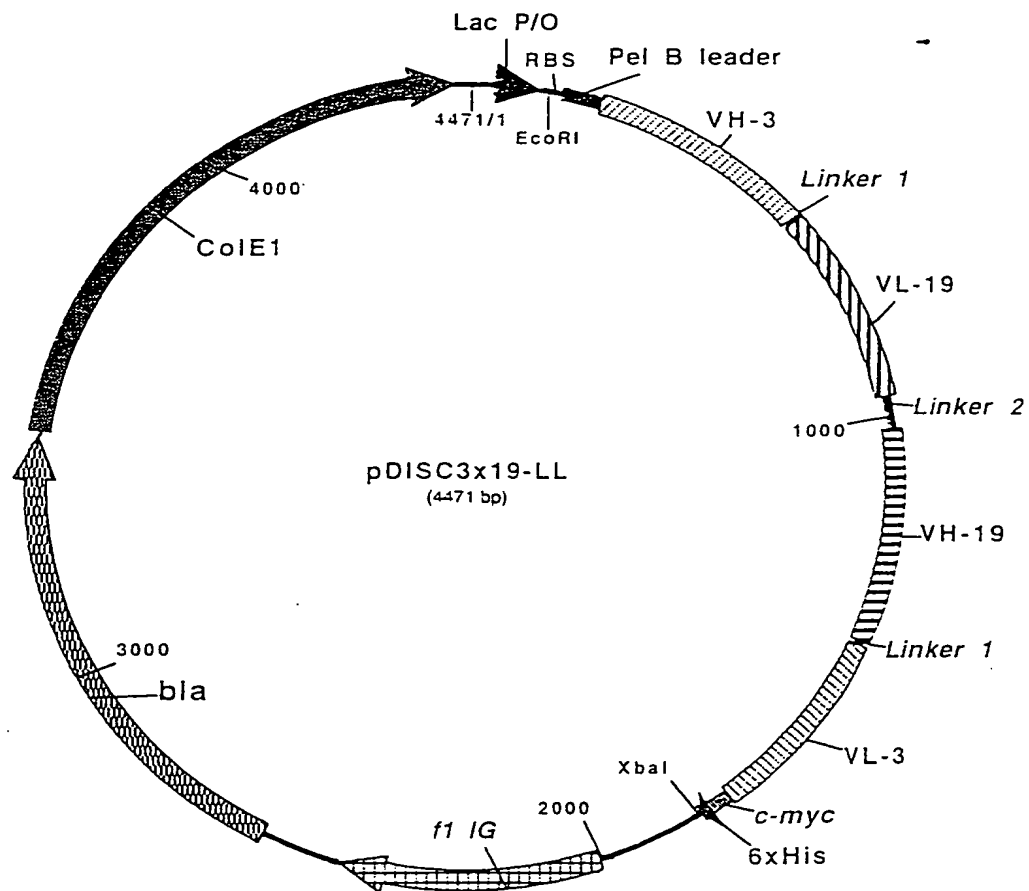


FIGURE 3

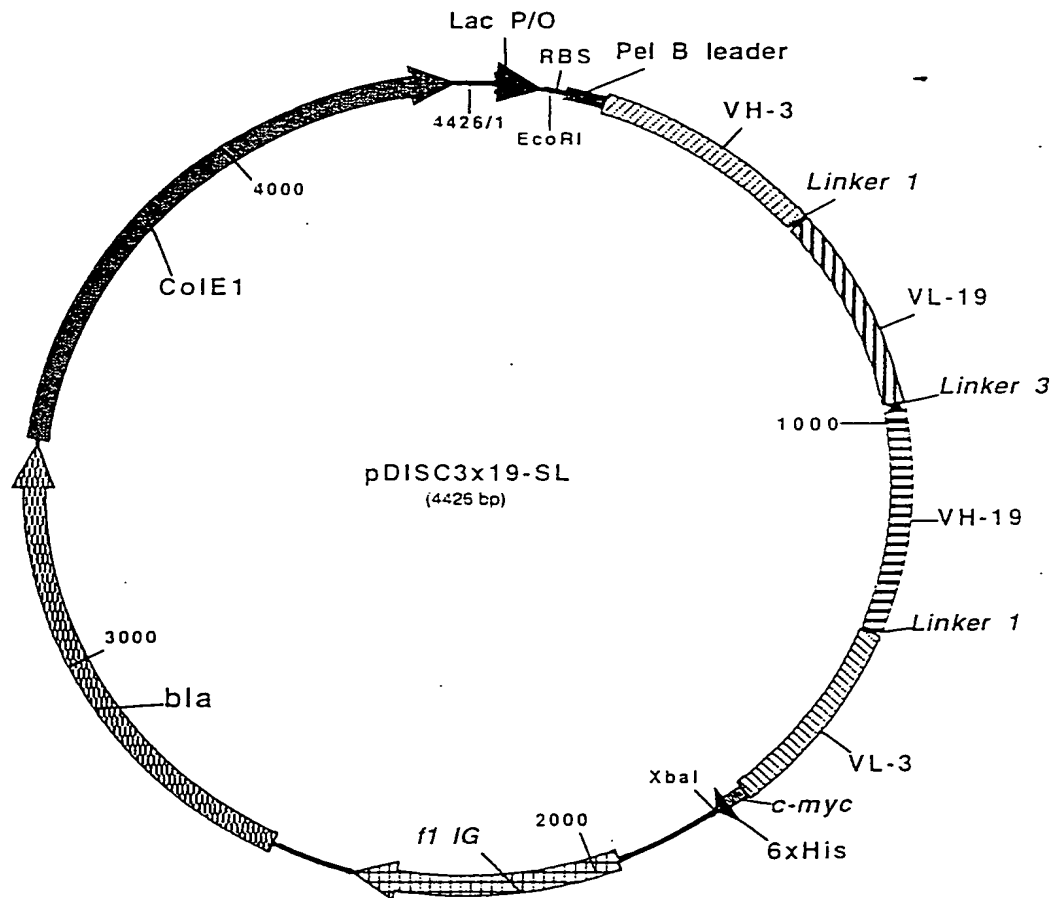


FIGURE 4

EcoRI RBS PstI leader NcoI

1 GAATTCATTAAAGGGAATTAACCATGAATACCTATTGCTTACGGCAGCCGCTGGCTTGTCTGCTGCTGGCGCTCAGCGGGCCATGC

12 M K Y L L P T A A A G L L L L A A Q P A M

Frame-H1 VH anti-CD3

92 CCAGGTGCAACTGCAGCAGTCTCGGGCTGAAGTGGCAAGACCTGGGGCTCAGTGAAGATGTCTGCAAGGCTTCTGGCTACACCTTTAC

22 A Q V Q L Q Q S G A E L A R P G A S V K M S C A K A S G Y T F T

CDR-H1 Frame-H2 CDR-H2

183 TAGGTACACGATGCACCTGGTAAACAGAGGCTGGACAGGCTCTGGAATGGATTGGATACATTAACTCTAGCCGCTGGTTATAC

52 R Y T M H W V K Q R P G Q G L E W I G Y I N P S R G Y T

Frame-H3

257 TAATTACAATCAGAAGTTCAAGGACAGGGCCACATTGACTACAGACAAATCTCCAGCACAGCCTACATGCACTGAGCGGCTGAC

30 N Y N Q K F K D K A T L T T D K S S S T A Y M Q L S S L T

Frame-H4

354 ATCTGAGGACTCTGCACTCTATTACTGTGCAAGATATTATGATGATCATTAACAGCCTTGACTACTGGGCGGAGGCCACTCTCA

109 S E D S A V Y Y C A R Y Y D D H Y S L D Y W G Q G T T L

CH1 Linker 1 Frame-L1 VL anti-CD19

440 CAGTCTCTCTAGCCCAACACCCCAAGCTTGGCGGTGATATCTTGTCTACCCAACTCCAGCTTCTTTGGCTGTGTCTTAGGGCAGA

158 T V S S A K T T P K L G G D I L L T Q T P A S L A V S L G Q

CDR-L1 Frame-L2

530 GGGCCACCATCTCTGCAAGGCCAGCCAAAGTGTGATTATGATGGTGATAGTTATTTGAACTCGTACCAACGATTCCAGGAC

158 R A T I S C K A S Q S V D Y D G D S Y L N W Y Q Q I P G

CDR-L2 Frame-L3

614 AGCCACCCAACTCTCTATGATGTCATCCAACTCTAGTTTCTGGGATCCCAAGCTTTAGTGGCAGTGGGTCTGGGACAGACTT

156 Q P P K L L I Y D A S N L V S G I P P R F S G S G S G T D F

CDR-L3 Frame-L4

702 CACCTCAACATCCATCTCTGTGGGAAGGTGGATGCTGCAACCTATCTCTCAGCAAAGTACTGAGGATCCCTGGACCTTCGGGA

225 T L N I H P Y E K V D A A T Y H C Q Q S T E D P W T F G G

C kappa NotI Linker 2

790 GGCACCAAGCTGGAATCAAAAGGCTGGGCGGCTGGTGGTGGTGGTCTGGCGGGGGTGGTAGCGGTGGTGGCGGG

255 G T K L E I K R A D A A A G G G G S G G G S G G G G

PvuII Frame-H1 VH anti-CD19

874 TCCGGTGGTGGTGGTAGCCAGGTGCAGTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCTGGGCTCTCAGTGAAGATTCTCTCAAGG

283 S G G G G S Q V Q L Q Q S G A E L V R P G S S V K I S C K

CDR-H1 Frame-H2 CDR-H2

962 CTTCTGGCTATGCATTCAGTACCTACTGGATGAACTGGGTGAAGCAGAGGCTGGACAGGCTCTTCACTGGATGGACAGATTGGG

312 A S G Y A F S S Y W M N W V K Q R P G Q G L E W I G Q I W

PstI Frame-H3

1049 CTGGAGATGGTGATACTAACTACAATGGAAGTTCAAGGGTAAAGCCACTCTGACTGCAGACCAATCTCCAGCACGCTTACA

341 P G D G D T N Y N G K F K G K A T L T A D E S S S T A Y

CDR-H3

1133 TGCACCTCAGCAGCCTAGCATCTGAGGACTCTGGGCTCTATTCTGTGCAAGACGGGAGACTACGACGGTAGGCCGTTATTACTAT

369 M Q L S S L A S E D S A V Y F C A R R E T T T V G R Y Y Y

Frame-H4 CH1 Linker 1 Frame-L1

1219 GCTATGGACTACTGGGGTCAAGGACCTCAGTCAAGCTCTCTCAGCCCAACACACCCCAAGCTTGGCGGTGATATCTGCTCACTC

398 A M D Y W G Q G T S V T V S S A K T T P K L G G D I V L T

VL anti-CD3 CDR-L1 Frame-L2 CDR-L2 Frame-L3

1307 AGTCTCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTACCATGACTGCAGTGGCCAGCTCAAGTGTAAGTTACATGAACTGG

427 Q S P A I M S A S P G E K V T M T C S A S S S V S Y M N W

Frame-L2 CDR-L2 Frame-L3

1393 TACCGCAGAAGTCAGGCACCTCCCCCAAGATGGATTTATGACACATCCAAAAGTGGCTTCTGGAGTCCCTGCTCACTTCAGGGGCA

456 Y Q Q K S G T S P K R W I Y D T S K L A S G V P A H F R G

CDR-L3

1481 GTGGGCTGGGACCTCTTACTCTCTCAATCAGCGGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGAGTAGTAA

485 S G S G T S Y S L T I S G M E A E D A A T Y Y C Q Q W S S N

Frame-L4 C kappa c-myc epitope

1569 CCGATTACAGTTCGGGCTCGGGGACAAAGTTGGAATAAAGCTGCTGCTCCAACTGGATCCGAACAAAAGCTGATCTCAG

514 P F T F G S G T K L E I N R A D T A P T G S E Q K L I S

His6 tail XbaI

1655 AAGAAGACCTAAACTCACATCCATCCATCACTCAATCTAGA

543 E E D L N S H H H H H H

FIGURE 5

6/10

EcoRI RBS PelB leader NcoI

1 GAATTCTTAAAGAGGGAATTAACCATGAAATACCTATTGCTTACGGGACGGCGTGGCTTGGCTGCTGCTGGCAGCTCAGCCGGCCATGG

1) M K Y L L P T A A A G L L L L A A Q P A M

Frame-H1 VH anti-CD3

92 CGCAGGTGCAACTGCAGCAGTCTGGGGCTGAAGTGGCAAGACCTGGGGCTCAGTGAAGATGTCTGCAAGGCTTCTGGCTACACCTTTAC

22) A Q V Q L Q Q S G A E L A R P G A S V K M S C K A S G Y T F T

CDR-H1 Frame-H2 CDR-H2

183 TAGGTACACGATGCACCTGGGTAAACAGAGGCTGGACAGGGCTCGAATGGATTGGATACATTAATCCCTAGCCGTGGTTATAC

52) R Y T M H W V K Q R P G Q G L E W I G Y I N P S R G Y T

Frame-H3

257 TAATTACAATCAGAAGTTCAAGGACAGGCCACATTCAGACAAATCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGAC

80) N Y N Q K F K D X A T L T T D K S S S T A Y M Q L S S L T

CDR-H3 Frame-H4

354 ATCTGAGGACTCTGCACTCTATTACTGTGCAAGATATTATGATGATCATTACAGCCTTGACTACTGGGGCCAGGCCACCTCTCA

109) S E D S A V Y Y C A R Y Y D D H Y S L D Y W G Q G T T L

CH1 Linker 1 Frame-L1 VL anti-CD19

440 CAGTCTCTCAGCCCAACACCAAGCTTGGCGGTGATATCTTGCTCACCCTCAAGCTTCTTTGGCTGTGTCTTAGGGCAGA

133) T V S S A K T T P K L G G D I L L T Q T P A S L A V S L S Q

CDR-L1 Frame-L2

530 GGGCCACCATCTCTGCAAGGCCAGCCAAAGTGTGTGATTATGATGGTGATAGTTATTTGAACTGGTACACAGATTCCAGGAC

168) R A T I S C K A S Q S V D Y D G D S Y L N W Y Q Q I P G

CDR-L2 Frame-L3

614 AGCCACCCAACTCTCTATCTATGATGGCATCCAACTAGTTTCTGGGATCCACCCAGGTTTAGTGGCAGTGGGTCTGGGACAGACTT

196) Q P P K L L I Y D A S N L V S G I P P R F S G S G S G T D F

CDR-L3 Frame-L4

702 CACCCCTCAACATCCATCTCTGTGGAGAGGTGGATGCTGCAACCTATCACTGTGAGCAAAGTACTGAGGATCCCTGGACGTTCGGTGA

225) T L N I H P V E K V D A A T Y H C Q Q S T E D P W T F G G

C kappa NotI Linker 3 PvuII Frame-H1

790 GGCACCAAGCTGGAATCAAAACGGGTGATGCTGGCGCGCTGGTGGCCCCAGGGTCGCAAGGTGCAAGTGCAGCAGTCTGGGGCTGAGCT

255) G T K L E I K R A D A A A A G G P G S Q V Q L Q Q S G A E L

VH anti-CD19 CDR-H1 Frame-H2

879 GGTGAGGCTGGGTCTCAGTGAAGATTTCCTGCAAGGCTTCTGGCTATGCATTGAGTACTGACTGGATGAACTGGGTGAAGCAGAGGC

284) V R P G S S V K I S C K A S G Y A F S S Y W M N W V K Q R

CDR-H2

968 CTGGACAGGGTCTTGAGTGGATTGCAAGATTGGCCCTGGAGATGGTGATACTAACTACAATGGAAAAGTTCAAGGGTAAAGCC

314) P G Q G L E W I G Q I W P G D G D T N Y N G K F K G K A

Frame-H3

1051 ACTCTGACTGCAGACGAATCTCTCCAGCAGCCTACATGCAACTCAGCAGCCTAGCATCTGAGGACTCTGGCGTCTATTCTGTGCAAGAC

342) T L T A D E S S S T A Y M Q L S S L A S E D S A V Y F C A R

CDR-H3 Frame-H4 CH1

1142 GGGAGACTACGACGGTAGGCCGTATTACTATGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCTCAAGCTAAA

372) R E T T T V G R Y Y A M D Y W G Q G T S V T V S S A K

Linker 1 Frame-L1 VL anti-CD3

1226 CAACACCAAGCTTGGCGGTGATATCTGCTCACTCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAGGTCACTGACTGCA

400) T T P K L G G D I V L T Q S P A I M S A S P G E K V T M T C

CDR-L1 Frame-L2 CDR-L2

1316 GTGCCAGCTCAAGTGTAAGTTACATGAAGTGTACAGCAGCAAGTCAGGCACCTCCCCAAAAGATGGATTATGACACATCCAA

430) S A S S S V S Y M N W Y Q Q K S G T S P K R W I Y D T S K

Frame-L3

1401 ACTGGCTTCTGAGTCCCTGCTCACTTCAGGGGCACTGGGTCTGGGACCTCTTACTCTCTCAATCAGGGCATGGAGGCTGAAGATGC

458) L A S G V P A H F R G S G S G T S Y S L T I S G M E A E D A

CDR-L3 Frame-L4 C kappa

1491 TGCCACTTATTACTGCAGCAGTGGAGTAGTAACCCATTTCAGCTTCGGCTCGGGGACAAAGTTGGAATAAACCAGGGCTGATCTGC

488) A T Y Y C Q Q W S S N P F T F G S G T K L E I N R A D T A

c-myc epitope His6 tail XbaI

1578 ACCCACTGGATCCGAACAAAAGCTGATCTCAGAAAGAACCTAAACTCAGTCACCATCAGCATCAATCTAGA

517) P T G S E Q K L I S E E D L N S H H H H H H H

FIGURE 6

941 ATGAGATTTTCCTTCAATTTTACTGCTGTTTTATTTCGCAGCATCCTCCGCATTAGCTGCTCCAGTCAACACTAC
1▶ M R F P S I F T A V L F A A S S A L A A P V N T T

alpha-factor signal

1015 AACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCATCGGTTACTCAGATTTAGAAGGGGATTTCGATG
25▶ T E D E T A Q I P A E A V I G Y S D L E G D F D

1089 TTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTTTTATAAATACTACTATTGCCAGCATTGCT
50▶ V A V L P F S N S T N N G L L F I N T T I A S I A

XhoI EcoRI

1163 GCTAAGAAGAAGGGGTATCTCTCGAGAAAAGAGAGGCTGAAGCTGAATTCCAGGTGCAACTGCAGCAGTC
75▶ A K E E G V S L E K R E A E A E F Q V Q L Q Q S

VH anti-CD3

1234 TGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCT
98▶ G A E L A R P G A S V K M S C K A S

FIGURE 7

941 ATGAGATTTTCCTTCAATTTTTACTGCTGTTTTATTTCGCAGCATCCTCCGCATTAGCTGCTCCAGTCAACACTAC
1> M R F P S I F T A V L F A A S S A L A A P V N T T

alpha-factor signal

1015 AACAGAAGATGAAACGGCACAAATTCCGGCTGAAGCTGTCAATCGGTACTCAGATTTAGAAGGGGATTTTCGATG
25> T E D E T A Q I P A E A V I G Y S D L E G D F D

BsrDI

1089 TTGCTGTTTTGCCATTTTCCAACAGCACAAATAACGGGTTATTGTTTATAAATACTACTATTGCCAGCATTGCT
50> V A V L P F S N S T N N G L L F I N T T I A S I A

XhoI

EcoRI

1163 GCTAAAGAAGAAGGGGTATCTCTCCAGAAAAAGAGAGGCTGAAGCTGAATTCATGGCGCAGGTGCAACTGCAG
75> A K E E G V S L E K R E A E A E F M A Q V Q L Q

VH anti-CD3

1235 CAGTCTGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGGCTTCT
99> Q S G A E L A R P G A S V K M S C K A S

FIGURE 8

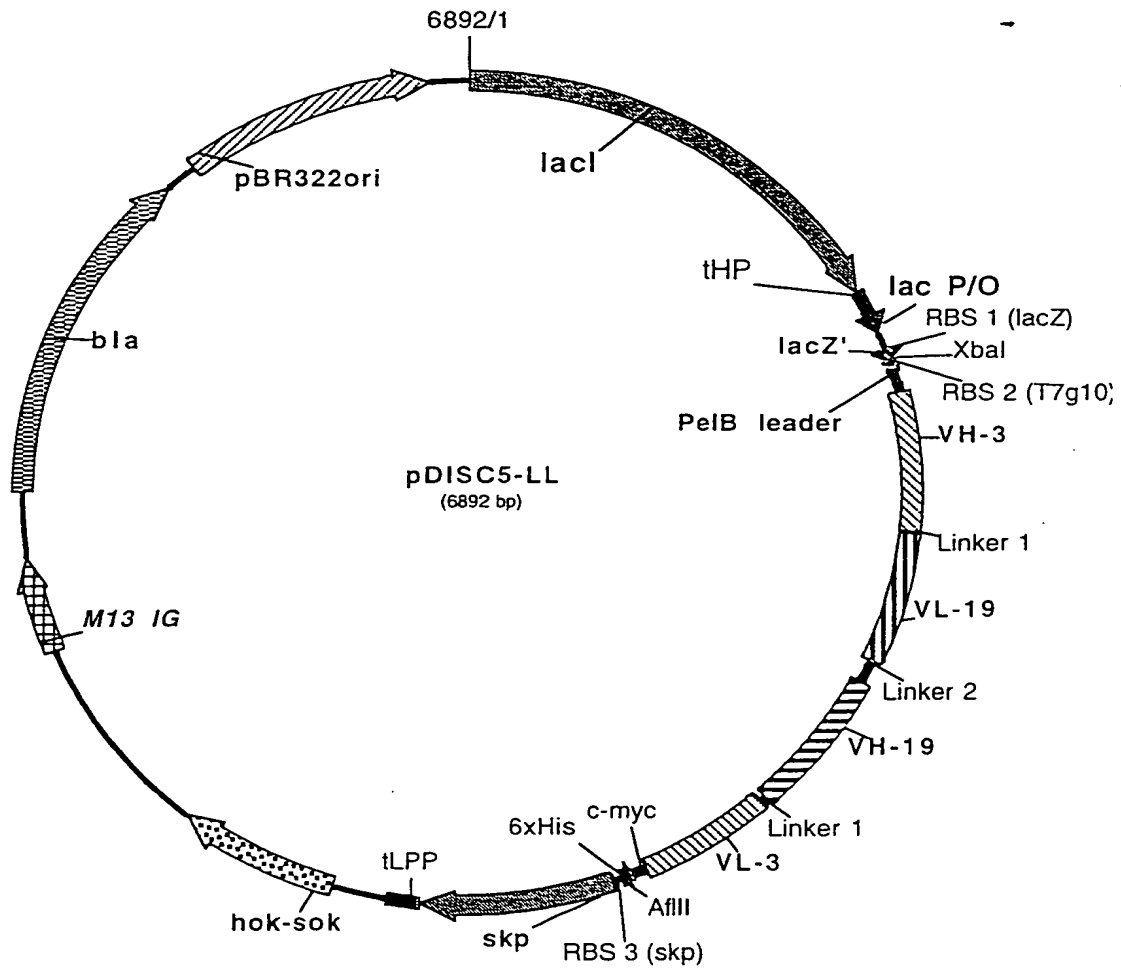


FIGURE 9

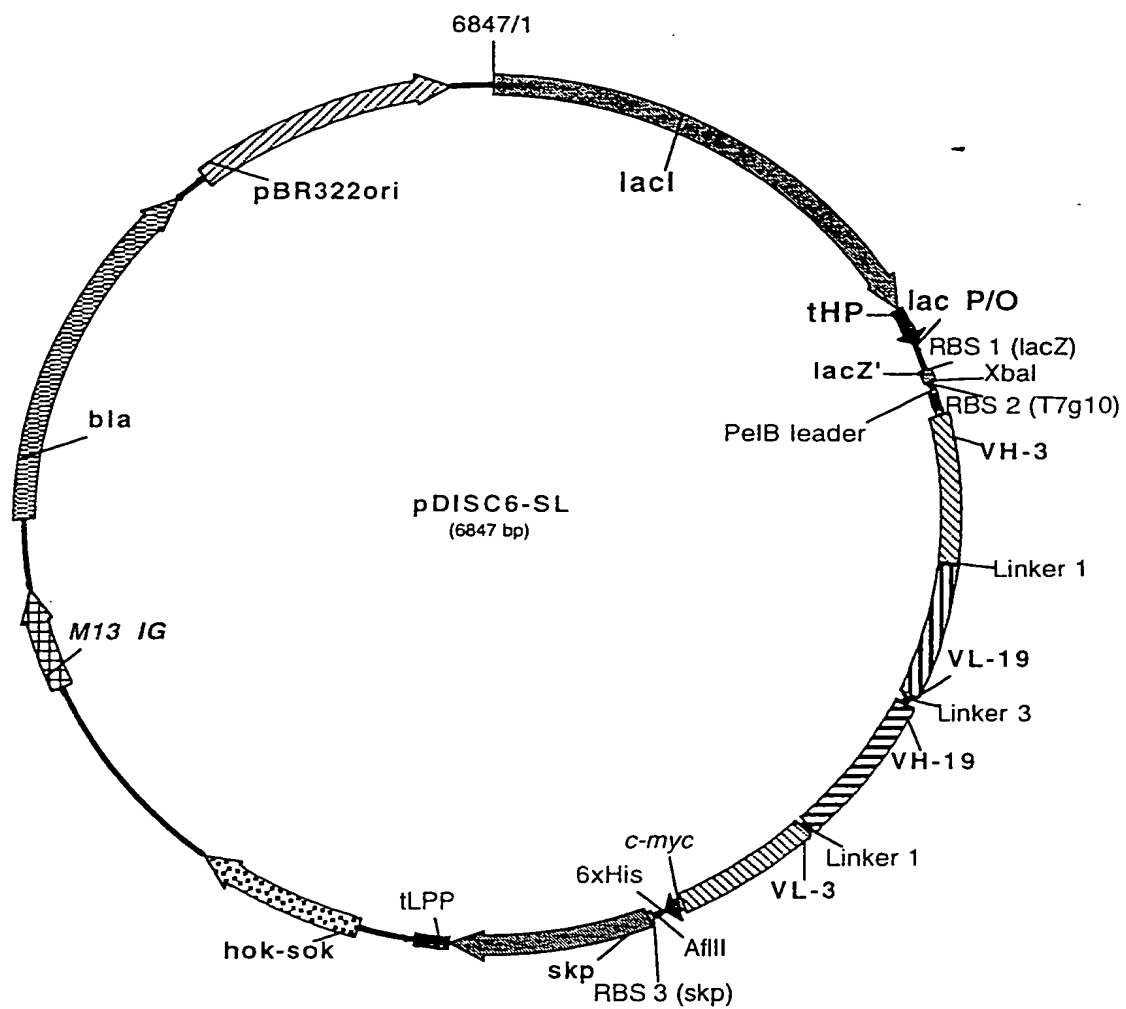


FIGURE 10